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Hepatitis C Virus Core Protein Fused to Hepatitis B Virus Core Antigen for Serological Diagnosis of Both Hepatitis C and Hepatitis B Infections by ELISA

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The sequence encoding the truncated core protein (amino acids 1–98) of hepatitis C virus (HCc) was expressed in *E. coli* for production of $HCc_{(1–98)}$, or fused with the truncated core antigen (HBcAg) and segments from the preS1 and preS2 regions from hepatitis B virus (HBV) for production of $HBcPreS_1PreS_2HCc_{(1–98)}$. The $HCc_{(1–98)}$ and $HBcPreS_1PreS_2HCc_{(1–98)}$ proteins reacted with sera from HCV-infected individuals by immunoblot analyses, while the latter protein also exhibited HBV core antigenicity. They induced antibodies against HBcAg and/or HCV core protein in rabbits and in mice. Moreover, $HBcPreS_1PreS_2HCc_{(1–98)}$ is more immunogenic than $HCc_{(1–98)}$ in terms of anti-HCc induction. An ELISA that employed recombinant HCV core antigens of either $HCc_{(1–98)}$ or $HBcPreS_1PreS_2HCc_{(1–98)}$ to detect anti-HCc and/or anti-HBc antibodies was developed. Evaluation of serum samples with different status of HBV and HCV infections suggested that $HCc_{(1–98)}$ might be suitable for the determination of antibodies against HCV core protein, while $HBcPreS_1PreS_2HCc_{(1–98)}$ might be of value to detect HCV and/or HBV infection in donated blood in HBV low-prevalence countries. ***J. Med. Virol. 57:104–110, 1999.*** © 1999 Wiley-Liss, Inc.

KEY WORDS: HCV core; HBV core antigen; hybrid HBcAg particle; diagnosis

INTRODUCTION

Hepatitis C virus (HCV) is a major cause of parenterally acquired viral non-A, non-B hepatitis [Kuo et al., 1989]. Over 60% of acute hepatitis C becomes chronic and may progress to cirrhosis and hepatocellular carcinoma [Saito et al., 1990]. Early diagnosis of hepatitis C is thus particularly important. In addition, accumulating data show that prompt interferon treatment can prevent the disease from becoming chronic [Davis et al., 1989; Di Bisceglie et al., 1989]. The demonstration of antibodies to HCV is an indication of prior or current infection with HCV. Recombinant proteins of high purity have been produced from various regions of the HCV genome and have been used singly or in combination in the serodiagnosis of antibodies against HCV structural and/or nonstructural proteins. The *C* gene of HCV consists of 191 codons, capable of encoding the putative core protein of 21 kD. Both B-lymphocyte and cytotoxic T-lymphocyte (CTL) determinants have been identified in the nucleocapsid [Nasoff et al., 1991; Koziel et al., 1993; Shirai et al., 1994]. As anti-core antibody is regarded as the earliest marker of seroconversion during HCV infection [Harada et al., 1991; Nasoff et al., 1991; Okamoto et al., 1992] and the IgM anti-core antibody may serve as a marker for acute or active HCV infection [Chen et al., 1992a], the core protein is of particular value in early diagnosis of hepatitis C.

The nucleocapsid protein or core antigen (HBcAg) of hepatitis B virus (HBV) can be produced efficiently in *E. coli* [Burrell et al., 1979; Stahl et al., 1982], where it self-assembles into core-like particles closely resembling those found in the liver of infected individuals [Cohen and Richmond, 1982]. Fusion of peptide sequences to HBcAg enhances their immunogenicity, probably because the HBcAg provides T-cell help [Milich et al., 1987a] and the fusion proteins retain the polymeric or particulate nature of HBcAg [Stahl and Murray, 1989]. HBcAg showed remarkable flexibility and high capacity in acceptance of long foreign insertions [Pumpens et al., 1995]. Peptide sequences of varying length have been fused to HBcAg at the N-terminus [Stahl et al., 1982; Clarke et al., 1987], the truncated

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C-terminus [Stahl and Murray, 1989; Schodel et al., 1992; Shiau and Murray, 1997], and some internal sites [Borisova et al., 1993; Schodel et al., 1994; Tindle et al., 1994] without affecting the self-assembly properties of HBcAg.

In the present study, the pRSET prokaryotic expression vector driven by the T7 RNA polymerase promoter [Kroll et al., 1993] was used to express the truncated N-terminal half (amino acids 1–98) of the HCV core protein alone. Due to the low expression level of the HCV core protein, it was also expressed as a fusion with HBcAg to facilitate expression and purification. An ELISA using the recombinant protein of either $HCc_{(1–98)}$ or $HBcPreS_1PreS_2HCc_{(1–98)}$ as the solid-phase antigen to detect anti-HCc and/or anti-HBc antibodies was developed. The results also suggest the potential value of $HBcPreS_1PreS_2HCc_{(1–98)}$ for screening of blood donors with either HCV or HBV, or both infections in HBV low-prevalence countries.

MATERIALS AND METHODS

Bacterial Strains and Plasmids

All plasmids constructed were propagated in *E. coli* strain TG1 or DH5α. For protein overexpression, *E. coli* strain BL21(DE3)pLysS bearing the T7 RNA polymerase gene was used. Plasmids pRSET-B [Kroll et al., 1993] and $pHBcPreS_1PreS_2S$ [Shiau, 1993] have been described. The plasmid pUC-9195 containing the coding sequence for the first 160 amino acid residues of the core protein from the HCV-B strain [Chou et al., 1993] was a gift from W.H. Chou.

Construction of Expression Plasmids

A cDNA fragment of 0.3 kb encompassing the amino-terminal 98 amino acids of HCc was obtained by the polymerase chain reaction (PCR) using pUC-9195 as the template and two oligonucleotides, HCV-7 (5′-CGGGATCCCATGAGCACGAATCCTAAACC) and HCV-8 (5′-GGAATTCTTACAGGAGCCATCCTGCCCA), as primers. The PCR product was digested with *Bam*HI and *Eco*RI, and inserted between the *Bam*HI and *Eco*RI sites of the pRSET-B. The resulting plasmid, $pRSET\text{-}HCc_{(1–98)}$, directs the expression of the truncated HCV core protein $HCc_{(1–98)}$.

To construct the vector expressing the HBcAg fusion protein with the HCV core protein, a similar cDNA fragment encompassing amino acids 1–98 of the HCV core protein was obtained by PCR using pUC-9195 as the template and with a set of primers: HCV-1 (5′-ACGCGTCGACATGAGCACAAATCCTAAAC) and HCV-2 (5′-ACGCGTCGACCAGGAGCGATCCTGCCCA). The PCR product was digested with SalI and fused to $pHBcPreS_1PreS_2S$ that had been digested with *Sal*I to release the $HBs_{(111–156)}$ fragment. The coding sequence of the HBcAg fusion protein was obtained by PCR using the resulting plasmid $pHBcPreS_1PreS_2HCc_{(1–98)}$ as the template with a pair of primers—HBC-3 (5′-CGGGATCCCATTGACCCTTATAAAGAATTT) and HCV-8—followed by digestion with *Bam*HI and *Eco*RI, and subcloned into *Bam*HI/*Eco*RI-digested pRSET-B expression vector. The resulting plasmid $pRSET\text{-}HBcPreS_1PreS_2HCc_{(1–98)}$ directs the expression of the HBcAg fusion protein carrying the truncated $HBcAg_{(3–144)}$, $PreS_{1(1–20)}$, and $PreS_{2(1–26)}$, as well as $HCc_{(1–98)}$ fragments.

The nucleotide sequences of the junction region in the constructs were verified by DNA sequencing. All vector constructions and analyses were carried out using standard techniques.

Production and Purification of Recombinant Proteins

Cultures of *E. coli* strain BL21(DE3)pLysS harboring various recombinant plasmids were grown and isopropyl-D-thiogalactopyranoside (IPTG, 0.5 mM) was added for induction of expression for 8 hr. The cells were harvested and lysed by sonication, and the cellular debris removed. Aliquots of cell lysate were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting with serum samples positive for anti-HCV antibody using standard procedures. The $HCc_{(1–98)}$ protein was purified with nickel-NTA-agarose affinity resin (Qiagen, Hilden, Germany) as described previously [Kroll et al., 1993]. The $HBcPreS_1PreS_2HCc_{(1–98)}$ was purified by ultracentrifugation followed by gel filtration on Sepharose 4B as described previously [Shiau and Murray, 1997].

Immunoblotting

After separation by SDS-PAGE, the proteins were electroblotted onto a nitrocellulose filter. The strips of the filter were treated with the blocking buffer (Tris-saline with 5% nonfat dry milk) for 2 hr at room temperature, then incubated with 500- to 1,000-fold diluted human serum positive for anti-HCV antibody or rabbit serum positive for anti-HBc antibody. The filter was washed with Tris-saline, then incubated with alkaline phosphatase-conjugated goat anti-human or goat anti-rabbit IgG. Reaction with 5-bromo-4-chloro-3-indolyl-phosphate and nitro blue tetrazolium was undertaken to detect the signal.

Animal Inoculations

Outbred New Zealand white male rabbits (2.5 kg) and inbred female BALB/cAnNCrj mice (6–8 weeks old) were immunized with $HCc_{(1–98)}$ or $HBcPreS_1PreS_2HCc_{(1–98)}$ emulsified in complete Freund's adjuvant (CFA) at doses of 1.5 mg intramuscularly (IM) and 100 μg intraperitoneally, respectively. They were boosted with the same antigen (1 mg for rabbits and 50 μg for mice) emulsified in incomplete Freund's adjuvant (IFA) after 21 days.

Serum Samples

Serum samples were collected from family members of patients with hepatocellular carcinoma, and exhibit various serological profiles in anti-HCV antibody and/or HBsAg. HBsAg was determined by the AUSRIA II-125 (Abbott Laboratories, North Chicago, IL) and anti-HCV antibody by the HCV EIA second generation (Abbott Laboratories).

The tests were double-checked and samples in the borderline reaction were excluded. They include anti-HCV(−)/HBsAg(−), anti-HCV(−)/HBsAg(+), anti-HCV(+)/HBsAg(−), and anti-HCV(+)/HBsAg(+) sera with 30 samples in each group for this study. The samples were further tested for HCV RNA by the reverse transcriptase-polymerase chain reaction (RT-PCR) using a set of nested primers from HCV 5′ noncoding region [Young et al., 1993] and for anti-HBc antibody by the Anticorase B-96 ELISA kit (General Biologicals Corp., Taiwan). Routine blood tests for liver functions were carried out, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The mean ALT and AST levels for the anti-HCV(+)/HBsAg(−) and anti-HCV(+)/HBsAg(+) groups were higher and in a wider range than those for the anti-HCV(−)/HBsAg(−) and anti-HCV(−)/HBsAg(+) groups that were in the normal range (data not shown).

ELISA

To evaluate the recombinant fusion proteins as antigens in an indirect ELISA, microtiter wells with high protein binding (Nunc, Roskilde, Denmark) were coated with $HCc_{(1–98)}$, HBcAg, or $HBcPreS_1PreS_2HCc_{(1–98)}$ (1 μg per well) in the coating buffer (carbonate-bicarbonate buffer, 0.05 M, pH 9.6), and free binding sites on the plastic were blocked with 10% calf serum in PBS. The plates were washed and incubated with various concentrations of serum samples diluted in PBS/10% calf serum from immunized animals or from human serum samples (1:100 dilution) with various status of HCV and/or HBV infections. Following incubation for 2 hr at 37°C, plates were re-washed and alkaline-phosphatase–conjugated goat anti-rabbit, -mouse, or -human antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) was added. After a further 2-hr incubation at 37°C, the plates were washed and developed with p-nitrophenyl phosphate. The enzyme reaction was stopped after 30 min with 0.1-M EDTA and the absorption measured at 405 nm. The data for antibody responses in immunized animals are expressed as antibody titer representing the highest dilution of serum required to yield four times of A_{405} of preimmune sera. For human serum samples, the cutoff value was arbitrarily calculated from the A_{405} as A_{405} of negative control + $(a) \times A_{405}$ of positive control. The value of a was assigned as 0.25 and 0.125 in the assay using $HCc_{(1–98)}$ and $HBcPreS_1PreS_2HCc_{(1–98)}$ as the solid-phase antigen, respectively.

Statistical Analysis

Chi-squared analysis with Yates' correction [Motulsky, 1995] where appropriate were used to compare frequencies of integers between two variables. Comparison of values of the A_{405} in the ELISA between groups was made using the Kruskal-Wallis test [Motulsky, 1995] for nonparametric data. Positive predictive value (PPV = number of true positive/number of true positive + number of false positive) and negative predictive value (NPV = number of true negative/number of true negative + number of false negative) were calculated [Motulsdky, 1995].

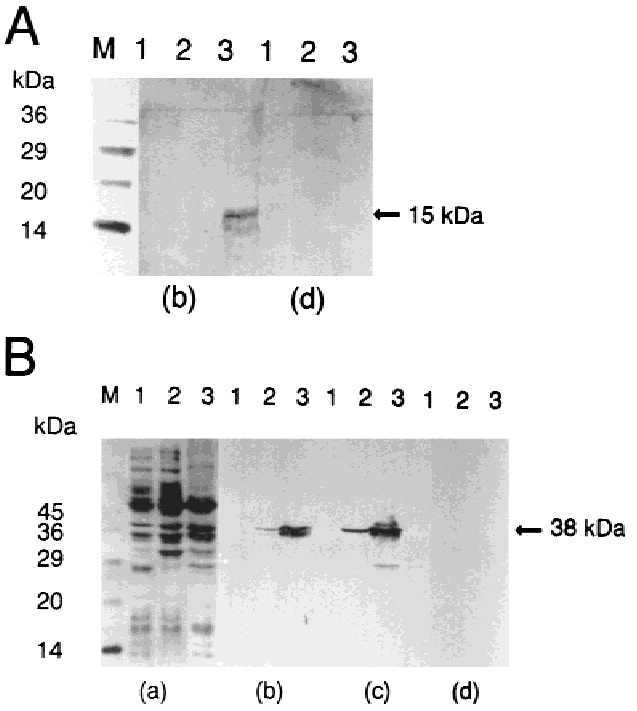

Fig. 1. SDS-PAGE and/or Immunoblot analysis of **(A)** $HCc_{(1–98)}$ and **(B)** $HBcPreS_1PreS_2HCc_{(1–98)}$ proteins from soluble fraction of crude extracts from (1) *E. coli* cultures harboring no plasmids, and from (2) uninduced and (3) IPTG-induced cultures harboring recombinant plasmids. (a) Detection on a Coomassie-stained 12.5% SDS-PAGE. (b) Immunoblot analysis probed with anti-HCV/EIA–positive patient's serum. (c) Immunoblot analysis probed with rabbit anti-HBc polyclonal antibody. (d) Immunoblot analysis probed with normal human serum.

RESULTS

Expression and Characterization of $HCc_{(1–98)}$ and $HBcPreS_1PreS_2HCc_{(1–98)}$

Both $HCc_{(1–98)}$ (15 kD) and $HBcPreS_1PreS_2HCc_{(1–98)}$ (38 kD) were found in the soluble fraction of the cell lysate after IPTG induction. However, $HBcPreS_1PreS_2HCc_{(1–98)}$ was also spontaneously produced in a lesser extent. In immunoblot analysis, $HCc_{(1–98)}$ and $HBcPreS_1PreS_2HCc_{(1–98)}$ gave strong positive reactions with serum samples positive for anti-HCV antibody, but not with normal control sera (Fig. 1A and B). Moreover, $HBcPreS_1PreS_2HCc_{(1–98)}$ also reacted with polyclonal anti-HBc antiserum (Fig. 1B).

Immunogenic Properties of $HCc_{(1–98)}$ and $HBcPreS_1PreS_2HCc_{(1–98)}$

Sera collected from immunized animals were subjected to adsorption by extracts from *E. coli* host cells at 4°C overnight followed by centrifugation to remove antibodies against *E. coli* proteins present in the serum samples. The animals immunized with $HCc_{(1–98)}$ elicited anti-$HCc_{(1–98)}$ antibodies (Fig. 2), while those immunized with $HBcPreS_1PreS_2HCc_{(1–98)}$ elicited antibodies against $HCc_{(1–98)}$, $HBcPreS_1PreS_2HCc_{(1–98)}$,

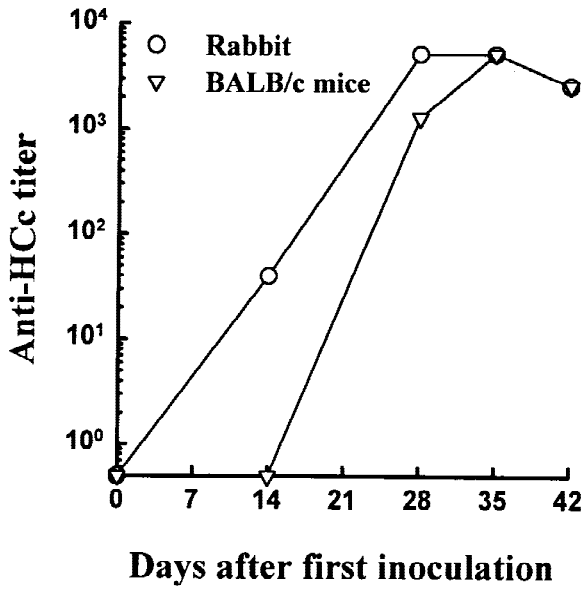

Fig. 2. Anti-HCc titers in animals immunized with $HCc_{(1–98)}$. A rabbit and four BALB/c mice were immunized with 1.5 mg and 100 μg, respectively, of $HCc_{(1–98)}$ in CFA on day 0, and boosted with 1 mg and 50 μg, respectively, in IFA on day 21. Sera were collected at time intervals and/or pooled from four mice for ELISA using $HCc_{(1–98)}$ as the solid-phase ligand. The data are expressed as antibody titer representing the highest dilution of serum required to yield four times the A_{405} of preimmune sera.

and, to a lesser extent, HBcAg (Fig. 3A and B). Notably, BALB/c mice elicited a high-titer of antibodies against the HBcAg fusion protein (Fig. 3B).

ELISA

Antibody reactivities to $HCc_{(1–98)}$ were analyzed with the ELISA in four groups of serum samples with anti-HCV(−)/HBsAg(−), anti-HCV(−)/HBsAg(+), anti-HCV(+)/HBsAg(−), and anti-HCV(+)/HBsAg(+), determined by Abbott HCV EIA and AUSRIA II-125 radioimmunoassay (RIA). Figure 4A shows the distributions and mean of A_{405} value in each group of serum samples with different anti-HCV/HBsAg profiles, while Figure 4B shows those grouped by reactivity with an HCV EIA. The A_{405} in serum samples negative for anti-HCV antibody detected by EIA is lower than that in serum samples positive for anti-HCV antibody. A cutoff value was set at 0.21. Among 120 serum samples, there were 52 and 50 samples positive and negative by both anti-$HCc_{(1–98)}$ ELISA and EIA, respectively. However, 8 positive samples by EIA were negative by anti-$HCc_{(1–98)}$ ELISA, while 10 negative samples by EIA were positive by anti-$HCc_{(1–98)}$ ELISA. An agreement was found between our anti-$HCc_{(1–98)}$ ELISA and Abbott EIA in 102/120 (85%) samples. The correlation between the presence of IgG antibodies to the recombinant $HCc_{(1–98)}$ segment and the HCV EIA kit positivity is moderately high.

On the other hand, using $HBcPreS_1PreS_2HCc_{(1–98)}$ as the antigen probe to test the antibody responses in 120 serum samples from different serological profiles of anti-HCV antibody and HBsAg exhibits a good correlation to discriminate anti-HCV(−)/HBsAg(−) from anti-HCV(+)/HBsAg(−), anti-HCV(−)/HBsAg(+), or anti-HCV(+)/HBsAg(+) (Fig. 5). The antigen reacted strongly with HCV antibodies, but showed a weaker reactivity for HBc antibodies as the A_{405} value in anti-HCV(+)/HBsAg(−) sera was higher than that in anti-HCV(−)/HBsAg(+) sera, and the A_{405} value in anti-HCV(+)/HBsAg(+) sera was only slightly higher than that in anti-HCV(+)/HBsAg(−) sera. A cutoff value was set at 0.14 for the assay. Of the 30 Abbott-EIA–negative and AUSRIA-II–negative samples, 28 were negative in our ELISA using $HBcPreS_1PreS_2HCc_{(1–98)}$ as the antigen probe. Among 90 samples with either Abbott-EIA–positive or AUSRIA-II–positive, or with both positive, 83 samples were positive in the ELISA using $HBcPreS_1PreS_2HCc_{(1–98)}$ as the antigen probe. There is good agreement between our single ELISA using HBcAg fusion protein with the HCc segment and combined Abbott EIA and AUSRIA II-125 tests in 111/120 (92.5%) samples. McNemar's test [Motulsky, 1995] was then applied to compare the two immunoassays. Comparison of our ELISA using $HBcPreS_1PreS_2HCc_{(1–98)}$ as the antigen with the combined tests of Abbott EIA and AUSRIA II-125 for dual detection of HCV and HBV infections generated a chi-squared value of 0.248, and comparison of the same test with AUSRIA II-125 for detection of HBV infection generated a chi-squared value of 1.0. Neither is significant at the 5% level, indicating good correlation between the tests for detection of HBV and/or HCV infections.

DISCUSSION

The full-length and C-terminally truncated HCV core proteins have been expressed in *E. coli* [Chen et al., 1992a; Yoshikawa et al., 1992; Handschuh and Caselmann, 1995; Khanna and Ray, 1995; Milton et al., 1995; Trowbridge et al., 1996]. The cDNA corresponding to codons 1–98 of the *C* gene of HCV that we used was deduced from the HCV-B strain [Chou et al., 1993]. The HCV-B strain isolated from Taiwan is closely related to the Taiwanese HCV-T strain [Chen et al., 1992b] and the Japanese HCV-J1 strain [Takeuchi et al., 1990]. As the core protein has a high degree of homology between different strains of virus [Takeuchi et al., 1990; Okamoto et al., 1991], variation in sensitivity of screening assays for different genotypes should be minimal [Dhaliwal et al., 1996]. Since the major immunodominant epitopes within HCc mostly lie in the amino-terminal half and the antibodies directed against these epitopes appear early after HCV infection [Nasoff et al., 1991; Nakagiri and Ichihara, 1995], the $HCc_{(1–98)}$ expressed in *E. coli* with an amino terminal tag of six histidines should be sufficient for examining the anti-HCc responses.

The core fragments $HCc_{(6–77)}$ and $HCc_{(6–143)}$ have been fused to truncated HBcAg for detection of anti-HCc antibodies [Claeys et al., 1992]. Hybrid HBcAg particles with multiple copies of $HCc_{(1–180)}$ were also made, which showed the surface expression of both

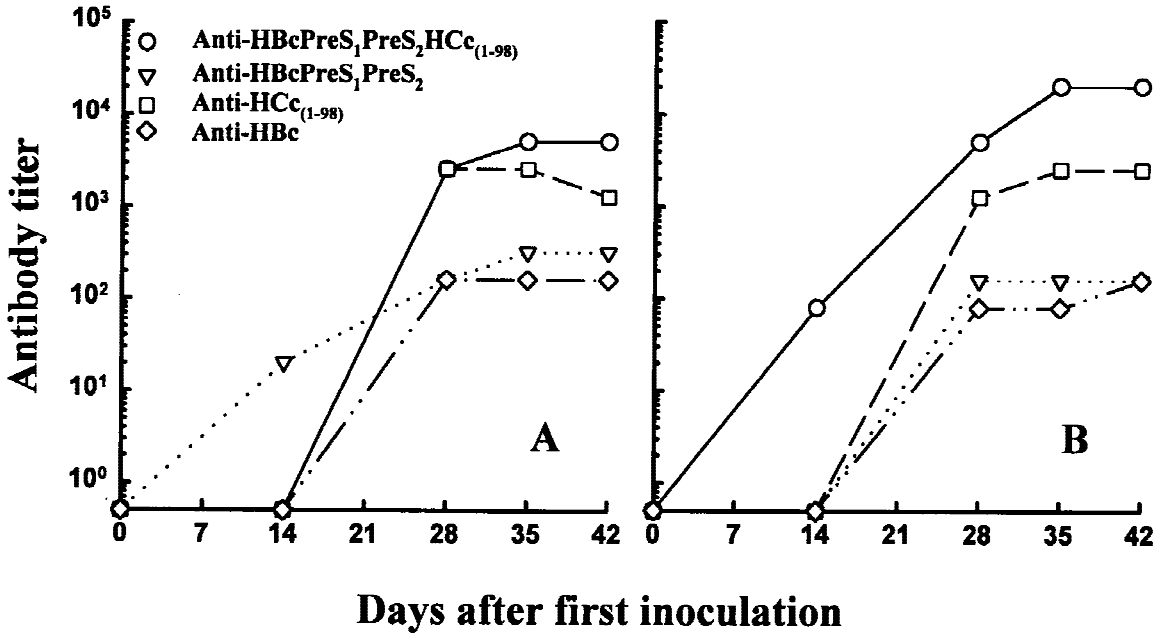

Fig. 3. Immunogenicity of $HBcPreS_1PreS_2HCc_{(1–98)}$ in animals. A rabbit **(A)** and four BALB/c mice **(B)** were immunized with 1.5 mg and 100 μg, respectively, in CFA on day 0, and boosted with 1 mg and 50 μg, respectively, in IFA on day 21. Sera were collected at time intervals and/or pooled from four mice for ELISA using $HBcPreS_1PreS_2HCc_{(1–98)}$, $HBcPreS_1PreS_2$, $HCc_{(1–98)}$, and HBcAg as the solid-phase ligand. The data are expressed as antibody titer representing the highest dilution of serum required to yield four times the A_{405} of preimmune sera.

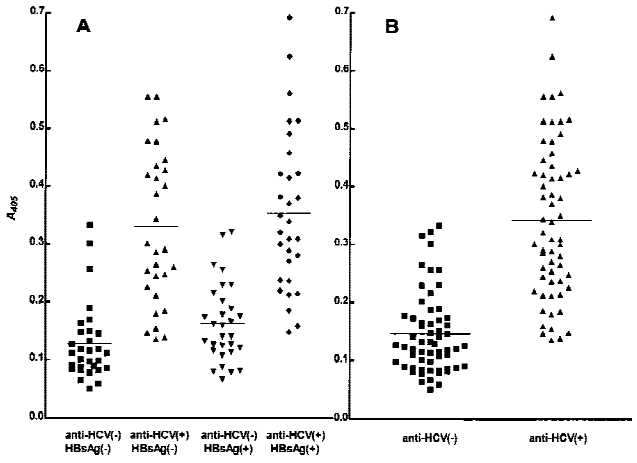

Fig. 4. Distribution of A_{405} in the ELISA using $HCc_{(1–98)}$ as the antigen probe in **(A)** four groups (n = 30) of serum samples with various serological profiles determined by the HCV EIA second-generation kit and the AUSRIA II-125, and in **(B)** two groups (n = 60) of serum samples positive or negative for anti-HCV determined by the HCV EIA second-generation kit. Mean values are indicated by horizontal bar.

HBV and HCV core epitopes [Yoshikawa et al., 1993]. In the work described here, the carrier moiety came from the truncated HBcAg with HBV $preS_{1(1–20)}$ and $preS_{2(1–26)}$ segments, which have been shown to contain B-cell and T-cell epitopes [Neurath et al., 1984; Milich et al., 1987b; Thornton et al., 1989]. Therefore, the $HBcPreS_1PreS_2HCc_{(1–98)}$ should be suitable for detecting antibodies to HCV core as well as to HBV core, preS1, and preS2.

In immunoblot analyses, both $HCc_{(1–98)}$ and $HBcPreS_1PreS_2HCc_{(1–98)}$ reacted with human sera positive for anti-HCV antibody. $HBcPreS_1PreS_2HCc_{(1–98)}$ also reacted with rabbit anti-HBc antiserum. These results confirm that the HCc sequence was presented in the HBcAg fusion particles containing the $HCc_{(1–98)}$ sequence. Antigenic reactivities of HBcAg and HCc were also analyzed by ELISA using serum samples positive for anti-HCc and/or HBsAg. We confirmed that all of the HBsAg-positive sera are anti-HBc positive. In the ELISA, $HBcPreS_1PreS_2HCc_{(1–98)}$ as the antigen probe reacted more strongly with sera positive for anti-HCV antibodies than with those positive for HBsAg. In the anti-HCV(–)/HBsAg(–) serum samples, 25 out of 30 samples are anti-HBc–positive. But only 2 out of these 25 samples showed reactivity with $HBcPreS_1PreS_2HCc_{(1–98)}$. Presumably the titers of anti-HBc in the HBsAg-negative sera may be lower than those in the HBsAg-positive sera, which rendered them undetectable by $HBcPreS_1PreS_2HCc_{(1–98)}$. This result also suggests that HBc antigenicity may be lower in $HBcPreS_1PreS_2HCc_{(1–98)}$than in the authentic HBcAg particle.

Sequences fused to the truncated C-terminal HBcAg are more likely to lie in troughs between the spikes of the nucleocapsid, or possibly within the core particle [Böttcher et al., 1997]. It is therefore intriguing that the HCc antigenicity is higher than HBc antigenicity displayed in the HBcAg fusion particle carrying the HCc sequence. It is possible that partial disassembly of the core may occur in the standard ELISA techniques based on support-bonded antigens. The conformation of the hybrid core particle may also be altered by the C-terminal insertion, resulting in lower HBc antigenicity.

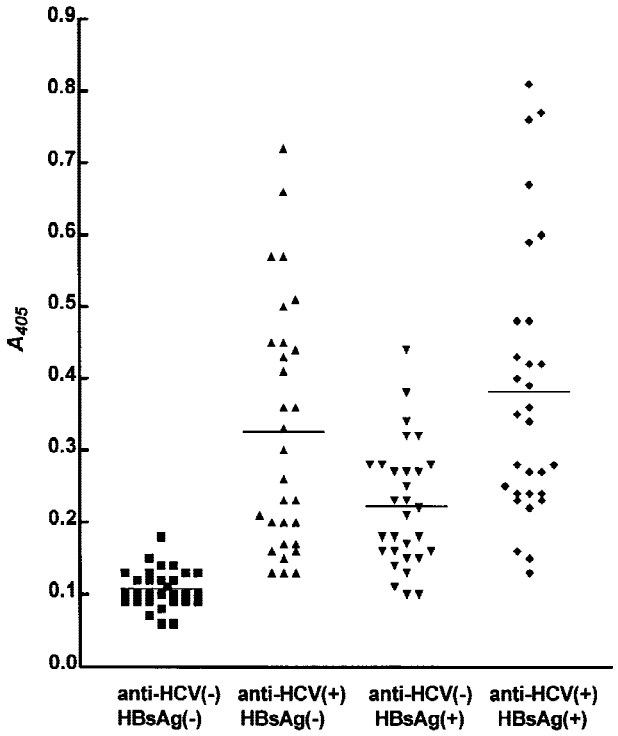

Fig. 5. Distribution of A_{405} in the ELISA using $HBcPreS_1PreS_2HCc_{(1–98)}$ as the antigen probe in four groups (n = 30) of serum samples with various serological profiles determined by the HCV EIA second-generation kit and the AUSRIA II-125. Mean values are indicated by horizontal bar.

Along this line, it has been shown that the truncated HBcAg fusion protein carrying $HCc_{(6–143)}$ in its C-terminus displayed higher HCc but lower HBc antigenicity, whereas in a similar fusion carrying $HCc_{(6–77)}$ the HBc antigenicity was similar to the authentic HBc protein [Claeys et al., 1992]. Therefore, it seems that the length and the sequence *per se* of the insert may influence the HBc antigenicity in the hybrid core particle.

The correlation between the presence of IgG antibodies to the truncated HCV core protein and the Abbott HCV EIA second-generation positivity is moderately high (85%). Moreover, when $HBcPreS_1PreS_2HCc_{(1–98)}$ was used as the antigen probe for the detection of either anti-HCc or anti-HBc antibodies, or both, the observed agreement between our ELISA test and combined tests by Abbott EIA and AUSRIA-II is 92.5%. The Abbott HCV EIA detects antibodies to HCV structural and nonstructural proteins, which also contain the core region, while AUSRIA II-125 detects HBV surface antigen. However, it is not possible to compare the sensitivity and specificity of our assays with the commercial diagnostic tests employed in this study, since a worldwide standard for assessing HCV infection is not yet available. Serum samples chosen for this study were all double-checked with Abbott HCV EIA and AUSRIA II-125 and those in the borderline reaction were excluded. Confirmation of HCV infection was also carried out by RT-PCR. Sixty initially negative sera for anti-HCV antibody were all HCV RNA-negative, whereas 40 initially positive sera were HCV RNA-positive. If we excluded 20 samples that were positive for Abbott HCV ELISA but were negative for HCV RNA from the 120 samples, the PPV and NPV are 78.26% and 92.59% for the ELISA using $HCc_{(1–98)}$ as the antigen probe in 100 serum samples confirmed by HCV RNA, respectively. For the ELISA using $HBcPreS_1PreS_2HCc_{(1–98)}$ as the antigen probe, PPV and NPV are 96.97% and 82.35%, respectively. Thus, with regard to the PPV, $HBcPreS_1PreS_2HCc_{(1–98)}$ performs better than $HCc_{(1–98)}$ as the antigen probe for ELISA. Moreover, there is good agreement between our single ELISA and combined Abbott EIA and AUSRIA II-125 tests. For screening of blood donors or many other purposes, it would be sufficient to use the hybrid HBcAg protein to detect either HCV or HBV, or both infections.

In conclusion, the N-terminal half of the HCV core protein was expressed as $HCc_{(1–98)}$ and $HBcPreS_1PreS_2HCc_{(1–98)}$ proteins using a prokaryotic expression system. An ELISA was set up using either the recombinant protein as the antigen probe to detect antibodies against the HCV core and/or HBV core proteins. In HBV endemic areas, the HBsAg(+) blood was excluded in the donated blood, while in the HBV low-prevalence countries, blood with any of the HBV markers, e.g., anti-HBs(+) and anti-HBc(+), is excluded. The $HBcPreS_1PreS_2HCc_{(1–98)}$ would be useful for detection of antibodies to HCV core and those to HBV core for the exclusion of blood contaminated with either or both HCV and HBV.

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REFERENCES

Borisova G, Arya B, Dislers A, Borschukova O, Tsibinogin V, Skrastina D, Eldarov MA, Pumpens P, Skryabin KG, Grens E. 1993. Hybrid hepatitis B virus nucleocapsid bearing an immunodominant region from hepatitis B virus surface antigen. J Virol 67: 3696–3701.

Böttcher B, Wynne SA, Crowther RA. 1997. Determination of the fold of the core protein of hepatitis B virus by electron cryomicroscopy. Nature 386:88–91.

Burrell CJ, Mackay P, Greenaway PJ, Hofschneider PH, Murray K. 1979. Expression in *Escherichia coli* of hepatitis B virus DNA sequences cloned in plasmid pBR322. Nature 279:43–47.

Chen PJ, Wang JT, Hwang LH, Yang YH, Hsieh CL, Kao JH, Sheu JC, Lai MY, Wang TH, Chen DS. 1992a. Transient immunoglobulin M antibody response to hepatitis C virus capsid antigen in posttransfusion hepatitis C: Putative serological marker for acute viral infection. Proc Nat Acad Sci USA 89:5971–5975.

Chen PJ, Lin MH, Tai KF, Liu PC, Lin CJ, Chen DS. 1992b. The Taiwanese hepatitis C virus genome: Sequence determination and mapping the 5′ termini of viral genomic and antigenomic RNA. Virology 188:102–113.

Chou WH, Sheu SH, Sheu L, Lu CF, Lin SY, Wu JS. 1993. Sequence analysis of nucleocapsid and partial envelope genes of the hepatitis C virus derived from an aboriginal asymptomatic carrier. Chinese J Microbiol Immunol 26:57–67.

Claeys H, Volckaerts A, Beenhouwer HD, Vermylen C. 1992. Association of hepatitis C virus carrier state with the occurrence of hepatitis C virus core antibodies. J Med Virol 36:259–264.

Clarke BE, Newton SE, Carroll AR, Francis MJ, Appleyard G, Syred AD, Highfield PE, Rowlands DJ, Brown F. 1987. Improved immunogenicity of a peptide epitope after fusion to hepatitis B core protein. Nature 330:381–384.

Cohen BJ, Richmond JE. 1982. Electron microscopy of hepatitis B core antigen synthesized in *E. coli*. Nature 296:677–679.

Davis GL, Balart LA, Schiff ER, Lindsay K, Bodenheimer HC Jr, Perrillo RP, Carey W, Jacobson IM, Payne J, Dienstag JL, VanThiel DH, Tamburro C, Lefkowitch J, Albrecht J, Meschievitz C, Ortego TJ, Gibas A, The Hepatitis Interventional Group. 1989. Treatment of chronic hepatitis C with recombinant interferon alfa: A multicenter randomized, controlled trial. Hepatitis Interventional Therapy Group. N Engl J Med 321:1501–1506.

Dhaliwal SK, Prescott LE, Dow BC, Davidson F, Brown H, Yap PL, Follett EAC, Simmonds P. 1996. Influence of viraemia and genotype upon serological reactivity in screening assays for antibody to hepatitis C virus. J Med Virol 48:184–190.

Di Bisceglie AM, Martin P, Kassianides C, Lisker-Melman M, Murray L, Waggoner J, Goodman Z, Banks SM, Hoofnagle JH. 1989. Recombinant interferon alfa therapy for chronic hepatitis C: A randomized, double-blind, placebo-controlled trial. N Engl J Med 321: 1506–1510.

Handschuh G, Caselmann WH. 1995. Bacterial expression and purification of hepatitis virus capsid proteins of different size. J Hepatol 22:143–150.

Harada S, Watanabe Y, Takeuchi K, Suzuki T, Katayama T, Takebe Y, Saito I, Miyamura T. 1991. Expression of processed core protein of hepatitis C virus in mammalian cells. J Virol 65:3015–3021.

Khanna A, Ray R. 1995. Hepatitis C virus core protein: Synthesis, affinity purification and immunoreactivity with infected human sera. Gene 153:185–189.

Koziel MJ, Dudley D, Afdhal N, Choo QL, Houghton M, Ralston R, Walker BD. 1993. Hepatitis C virus (HCV)-specific cytotoxic T lymphocytes recognize epitopes in the core and envelope proteins of HCV. J Virol 67:7522–7532.

Kroll DJ, Abdel-Malek Abdel-Hafiz H, Marcell T, Simpson S, Chen CY, Gutierrez-Hartmann A, Lustbader JW, Hoeffler JP. 1993. A multifunctional prokaryotic protein expression system: Overproduction, affinity purification, and selective detection. DNA Cell Biol 12:441–453.

Kuo G, Choo QL, Alter HJ, Gitnick GL, Redeker AG, Purcell RH, Miyamura T, Dienstag JL, Alter MJ, Stevens CE, Tegtmeier GE, Bonino F, Colombo M, Lee WS, Kuo C, Berger K, Shuster JR, Overby LR, Bradley DW, Houghton M. 1989. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science 244:362–364.

Milich DR, McLachlan A, Thornton GB, Hughes JL. 1987a. Antibody production to the nucleocapsid and envelope of the hepatitis B virus primed by a single synthetic T cell site. Nature 329:547–549.

Milich DR, McLachlan A, Moriarty A, Thornton GB. 1987b. A single 10-residue pre-S(1) peptide can prime T cell help for antibody production to multiple epitopes within the pre-S(1), pre-S(2), and S regions of HBsAg. J Immunol 138:4457–4465.

Milton ID, Watson JP, Guo K, Carter MJ, Bassendine MF, Toms GL. 1995. Prokaryotic expression and analysis of the antibody response to a Newcastle isolate of the core gene of hepatitis C. J Med Virol 45:253–258.

Motulsky H. 1995. Intuitive Biostatistics. New York: Oxford University Press.

Nakagiri I, Ichihara K. 1995. ELISA for anti-HCV antibody employing a shorter synthetic core region peptide. J Virol Methods 52: 195–207.

Nasoff MS, Zebedee SL, Inchauspe G, Prince AM. 1991. Identification of an immunodominant epitope within the capsid protein of hepatitis C virus. Proc Nat Acad Sci USA 88:5462–5466.

Neurath AR, Kent SB, Strick N. 1984. Location and chemical synthesis of a pre-S gene coded immunodominant epitope of hepatitis B virus. Science 224:392–395.

Okamoto H, Okada S, Sugiyama Y, Kurai K, Iizuka H, Machida A, Miyakawa Y, Mayumi M. 1991. Nucleotide sequence of the genomic RNA of hepatitis C virus isolated from a human carrier: Comparison with reported isolates for conserved and divergent regions. J Gen Virol 72:2697–2704.

Okamoto H, Sugiyama Y, Okada S, Kurai K, Akahane Y, Sugai Y, Tanaka T, Sato K, Tsuda F, Miyakawa Y, Mayumi M. 1992. Typing hepatitis C virus by polymerase chain reaction with type-specific primers: Application to clinical surveys and tracing infectious sources. J Gen Virol 73:673–679.

Pumpens P, Borisova GP, Crowther RA, Grens E. 1995. Hepatitis B virus core particles as epitope carriers. Intervirology 38:63–74.

Saito I, Miyamura T, Ohbayashi A, Harada H, Katayama T, Kikuchi S, Watanabe Y, Koi S, Onji M, Ohta Y, Choo QL, Houghton M, Kuo G. 1990. Hepatitis C virus infection is associated with the development of hepatocellular carcinoma. Proc Nat Acad Sci USA 87: 6547–6549.

Schodel F, Moriarty AM, Peterson DL, Zheng JA, Hughes JL, Will H, Leturcq DJ, McGee JS, Milich DR. 1992. The position of heterologous epitopes inserted in hepatitis B virus core particles determines their immunogenicity. J Virol 66:106–114.

Schodel F, Wirtz R, Peterson D, Hughes J, Warren R, Sadoff J, Milich D. 1994. Immunity to malaria elicited by hybrid hepatitis B virus core particles carrying circumsporozoite protein epitopes. J Exp Med 180:1037–1046.

Shiau AL. 1993. Immunological aspects of hepatitis B virus core antigen and its derivatives. PhD thesis. Edinburgh: University of Edinburgh.

Shiau AL, Murray K. 1997. Mutated epitopes of hepatitis B surface antigen fused to the core antigen of the virus induce antibodies that react with the native surface antigen. J Med Virol 51:159–166.

Shirai M, Okada H, Nishioka M, Akatsuka T, Wychowski C, Houghten R, Pendleton CD, Feinstone SM, Berzofsky JA. 1994. An epitope in hepatitis C virus core region recognized by cytotoxic T cells in mice and humans. J Virol 68:3334–3342.

Stahl S, MacKay P, Magazin M, Bruce SA, Murray K. 1982. Hepatitis B virus core antigen: Synthesis in *Escherichia coli* and application in diagnosis. Proc Nat Acad Sci USA 79:1606–1610.

Stahl SJ, Murray K. 1989. Immunogenicity of peptide fusions to hepatitis B virus core antigen. Proc Nat Acad Sci USA 86:6283–6287.

Takeuchi K, Boonmar S, Kubo Y, Katayama, T, Harada H, Ohbayashi A, Choo QL, Kuo G, Houghton M, Saito I, Miyamura T. 1990. Hepatitis C virus cDNA clones isolated from a healthy carrier donor implicated in post-transfusion non-A, non-B hepatitis. Gene 91:287–291.

Thornton GB, Moriarty AM, Milich DR, Eichberg JW, Purcell RH, Gerin JL. 1989. Protection of chimpanzees from hepatitis-B virus infection after immunization with synthetic peptides: Identification of protective epitopes in the pre-S region. In: Lerner RA, Ginsberg H, Chanock RM, Brown, F, editors. Vaccines 89: Modern approaches to new vaccines including prevention of AIDS. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory, p 467–471.

Tindle RW, Herd K, Londono P, Fernando GJ, Chatfield SN, Malcolm K, Dougan G. 1994. Chimeric hepatitis B core antigen particles containing B- and Th-epitopes of human papillomavirus type 16 E7 protein induce specific antibody and T-helper responses in immunised mice. Virology 200:547–557.

Trowbridge R, Sloots TP, Buda P, Faoagali J, Hyland C, Young I, Gowans EJ. 1996. An ELISA for the detection of antibody to the core antigen of hepatitis C virus: Use in diagnosis and analysis of indeterminate samples. J Hepatol 24:532–538.

Yoshikawa A, Takahashi K, Kishimoto S, Tsuda F, Akahane Y, Naito S, Tanaka T, Yoshizawa H, Yamasaki M, Okamoto H, Miyakawa Y, Mayumi M. 1992. Serodiagnosis of hepatitis C virus infection by ELISA for antibodies against the putative core protein ($p20^{e}$) expressed in *Escherichia coli*. J Immunol Methods 148:143–150.

Yoshikawa A, Tanaka T, Hoshi Y, Kato N, Tachibana K, Iizuka H, Machida A, Okamoto H, Yamasaki M, Miyakawa Y, Mayumi M. 1993. Chimeric hepatitis B virus core particles with parts or copies of the hepatitis C virus core protein. J Virol 67:6064–6070.

Young KC, Chang TT, Liou TC, Wu HL. 1993. Detection of hepatitis C virus RNA in peripheral blood mononuclear cells and in saliva. J Med Virol 41:55–60.